TABLE 10

BIOASSAYS USING *SPODOPTERA EXIGUA* LARVAE

Strain	Mutation	LC_{50}[1] (95% C. I.)[3]	LC_{95}[2] (95% C. I.)
EG11822	R148A	37 (32-43)[4]	493 (375-686)[4]
EG11832	R148D	22 (19-25)[4]	211 (167-282)[4]
Wild-type	None	145 (117-182)	1685 (1072-3152)
Mutant # 1	R148L	47 (39-57)	523 (367-831)
Mutant #12	R148G	65 (46-93)	549 (316-1367)
Mutant #43	R148L	31 (16-54)	311 (144-1680)
Mutant #45	R148M	36 (29-45)	469 (324-762)

[1]Concentration of Cry1C protein that causes 50% mortality expressed in ng crystal protein per 175 mm^2 well. Results of one set of replicated bioassays.

[2]Concentration of Cry1C protein that causes 95% mortality expressed in ng crystal protein per 175 mm^2 well. Results of one set of replicated bioassays.

[3]95% confidence intervals.

[4]Results of two sets of replicated bioassays.

5.5 EXAMPLE 5 -- SEQUENCE ANALYSIS OF *CRY1C* MUTATIONS

Recombinant plasmids from the EG10368 transformants were isolated using the alkaline lysis method (Maniatis *et al.*, 1982). Plasmids obtained from the transformants were introduced into the *E. coli* host strain DH5α™ by competent cell transformation and used as templates for DNA sequencing using the Sequenase® v2.0 DNA sequencing kit (U. S. Biochemical Corp., Cleveland, OH).

Sequence analysis of plasmid pEG359 (FIG. 4; SEQ ID NO:24) revealed the expected frameshift mutation at codon 118 and the *Bam*HI and *Bln*I restriction sites introduced by the mutagenic oligonucleotide primer B (SEQ ID NO:16).

Sequence analysis of the *cry1C.563* gene on plasmid pEG370 (FIG 4; SEQ ID NO:25) revealed nucleotide substitutions at positions 354, 361, 369, and 370, resulting in point mutations A to T, A to C, A to C, and G to A, respectively. These mutations

resulted in amino acid substitutions in Cry1C.563 (FIG. 4; SEQ ID NO:26) at positions 118 (E to D), 121 (N to H), and 124 (A to T).

Sequence analysis of the *cry1C.579* gene on plasmid pEG373 (FIG 4; SEQ ID NO:54) revealed nucleotide substitutions at positions 353, 369, and 371, resulting in point mutations A to T, A to T, and C to G, respectively. These mutations resulted in amino acid substitutions in Cry1C.579 (FIG. 4; SEQ ID NO:55) at positions 118 (E to V) and 124 (A to G).

Sequence analysis of the *cry1C.499* gene on plasmid pEG374 (FIG 4; SEQ ID NO:56) revealed nucleotide substitutions at positions 360 and 361, resulting in point mutations T to C and A to C, respectively. These mutations resulted in an amino acid substitution in Cry1C.499 (FIG. 4; SEQ ID NO:57) at position 121 (N to H).

Sequence analysis of the *cry1C* genes in EG11811 and EG11822 confirmed the substitution of alanine for arginine at position 148 (SEQ ID NO:1, SEQ ID NO:2). Nucleotide substitutions C442G and G443C yield the codon GCA, encoding alanine.

Sequence analysis of the random R148 mutants indicate changes of R148 to aspartic acid, methionine, leucine, and glycine. Thus, a variety of amino acid substitutions for the positively-charged arginine residue at position 148 in Cry1C result in improved toxicity. None of these substitutions can be regarded as conservative changes. Alanine, leucine, and methionine are non-polar amino acids, aspartic acid is a negatively-charged amino acid, and glycine is an uncharged amino acid, all possessing side chains smaller than that of arginine. All of these amino acids, with the exception of aspartic acid, differ significantly ($\pm$2 units) from arginine using the hydropathic and hydrophilicity indices described above.

The strain harboring the *cry1C-R148D* gene was designated EG11832. The nucleotide sequence of the *cry1C-R148D* gene is shown in SEQ ID NO:3, and the amino acid sequence is shown in SEQ ID NO:4. The nucleotide substitutions C442G, G443A, and A444C yield the codon GAC, encoding aspartic acid. The Cry1C-R148D mutant EG11832 exhibits a ~6.5-fold lower LC_{50} and a ~8-fold lower LC_{95} in bioassay against *S. exigua* when compared to the wild-type Cry1C strain.

5.6 EXAMPLE 6 -- SUMMARY OF *CRY1C** MUTANTS

The *cry1C* mutants of the present invention are summarized in Table 11.

TABLE 11

SUMMARY OF CRY1C* STRAINS

Cry1C Designation	Strain	Plasmid Name	Parental Plasmid
Cry1C.563	EG11740	pEG370	pEG916
Cry1C.579	EG11746	pEG373	pEG916
Cry1C.499	EG11747	pEG374	pEG916
Cry1C R148A	EG11811	pEG1635	pEG315
Cry1C R180A	EG11815	pEG1636	pEG315
Cry1C R148A	EG11822	pEG1639	pEG345
Cry1C R148D	EG11832	pEG1642	pEG345
Cry1C R148G	EG11833	pEG1643	pEG345
Cry1C R148L	EG11834	pEG1644	pEG345
Cry1C-R148A-K219A	EG12111	pEG1639	pEG1639
Cry1C-R148D-K219A	EG12121	pEG943	pEG1642
Cry1C R148M	EG11835	pEG1645	pEG345

5.7 EXAMPLE 7 -- CONSTRUCTION OF *B. THURINGIENSIS* STRAINS CONTAINING MULTIPLE *CRY* GENES IN ADDITION TO *CRY1C* AND *CRY1C R148A*

The *B. thuringiensis* host strain EG4923-4 may be used as a host strain for the native and mutant *cry1C* genes of the present invention. Strain EG4923-4 contains three *cry1Ac* genes and one *cry2A* gene on native plasmids and exhibits excellent insecticidal activity against a variety of lepidopteran pests. Recombinant plasmids containing the *cry1C* and *cry1C-R148A* crystal protein genes, originally derived from *aizawai* strain 7.29, were introduced into the strain EG4923-4 background using the electroporation procedure described by Mettus and Macaluso (1990). The recombinant plasmids containing *cry1C* and *cry1C-R148A* were designated pEG348 (FIG. 7) and pEG1641